

At page 5 of the Official Action, the Examiner has rejected claims 1-6 under 35 U.S.C. §103(a) as allegedly unpatentable over US Patent 5,811,238 to Stemmer et al. in view of Berger.

The rejections summarized above constitute the entirety of the rejections raised by the Examiner in the April 16, 2002 Official Action. No other issues are pending in the present application. Applicants respectfully submit that the claims as presently amended are in condition for allowance. Each of the above-noted rejections under 35 U.S.C. §§ 102 and 103 and the judicially created doctrine of obviousness-type double patenting is, therefore, respectfully traversed.

**CLAIMS 1-6 ARE NOT PROPERLY REJECTED UNDER THE JUDICIALLY
CREATED DOCTRINE OF OBVIOUSNESS DOUBLE PATENTING**

The Examiner has maintained the rejection of claims 1-6 under the judicially created doctrine of obviousness-type double patenting as allegedly unpatentable over claims 1-7 of U.S. Patent No. 6,159,690 ('690). The Examiner contends that, although claims 1-7 of the '690 patent are not identical, they are not patentably distinct. The Examiner has considered Applicants' arguments regarding the rejection of claim 1-6 under the doctrine of obviousness-type double patenting, but remains unpersuaded.

The Examiner contends that the template polynucleotide of the '690 patent could be a single-stranded or double-stranded oligonucleotide or a primer annealing to the target. The '690 patent discloses that the term "polynucleotide" refers interchangeably to both RNA and DNA. See column 3, lines 42-46. The skilled person appreciates that RNA is single stranded. However, this passage is silent regarding whether the specified DNA is single or double stranded. Applicants respectfully submit that the

Examiner is interpreting the silence in this passage with the hindsight accorded by the present invention.

In view of the amendment to claim 1 and dependent claims therefrom, Applicants assert that the Examiner's rejection of claims 1-6 under the judicially created doctrine of obviousness-type double patenting is improper. As amended, the language of claim 1 clearly omits the "template" recited in claims 1-7 of the '690 patent. Claim 1 has been further amended to clarify that the plus and minus single stranded populations are digested as separate populations and then contacted to facilitate annealing of complementary plus and minus strand fragments, a feature that is absent in the disclosure of the '690 patent. Support for this amendment can be found at page 20, lines 3-14 and in Figures 1-3 and the detailed descriptions thereof. Also see page 19, lines 15-20. The above passages in the specification describe the method steps of amended claim 1, including the feature of isolating two separate populations comprising either plus or minus single strand polynucleotides. Methodological details describing the production of the two separate single stranded populations are provided in the specification at page 24, line 21 over to page 26, line 4. The enzymatic digestion of the two single stranded populations as separate populations is described at page 26, lines 6-15. Conditions for contacting the two digested single stranded polynucleotide populations are provided at page 27, lines 10-21.

Claim 3 has been amended to depend from independent claim 1.

In view of the amendment to claim 1 and dependent claims therefrom, Applicants respectfully submit that the Examiner's rejection of claims 1-6 under the judicially created doctrine of obviousness-type double patenting based

on the '690 patent is inappropriate and respectfully urge that the rejection be withdrawn.

**CLAIMS 1-6 ARE NOT ANTICIPATED BY US PATENT 6,159,690 TO
BORREBAECK ET AL.**

It is well-established that in order to anticipate a claim, the cited reference must teach every element of the claim. As summarized in M.P.E.P. § 2131,

"A claim is anticipated only if **each and every element as set forth in the claim** is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). "**The identical invention** must be shown in as complete detail as is contained in the ... claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

In view of the above cited principles, Applicants respectfully submit that the claims as presently amended are not anticipated by the disclosure of the '690 patent to Borrebaeck et al.

The Examiner has maintained the rejection of claims 1-6 under 35 U.S.C. 102(b) as allegedly anticipated by the '690 patent issued to Borrebaeck et al. It is the Examiner's position that the '690 patent teaches each of the limitations found in claims 1-6. Furthermore, the Examiner contends that the open "comprising" format of these claims permits the inclusion of additional elements and thus, could encompass additional steps.

Applicants respectfully submit that the '690 patent fails to teach each of the features found in instant claims 1-6 as amended. The '690 application does not describe the generation of two separate populations of single stranded polynucleotide sequences, wherein the first population is comprised of plus strands of parent polynucleotide sequences and the second population is comprised of minus strands of parent polynucleotide sequences.

In the method of the instant application, these two separate plus and minus strand populations are separately digested with exonuclease to generate a population of plus strand fragments and a population of minus strand fragments. The minus and plus strand fragment populations are contacted and those capable of annealing are amplified to generate at least one polynucleotide sequence encoding one or more protein motifs having altered characteristics. Inasmuch as the present claims require the separate digestion of these single stranded populations, Applicants submit that the amendment to claim 1 serves to eliminate the inherency issue raised by the Examiner.

In light of all the foregoing, Applicants submit that claims 1-6 are novel over the disclosure of the '690 patent and respectfully request that the rejection under 35 U.S.C. §102(b) be withdrawn.

**CLAIMS 1-6 AS AMENDED ARE NOT RENDERED OBVIOUS BY US PATENT
5,811,238 TO STEMMER ET AL. IN COMBINATION WITH BERGER**

The Examiner has maintained the rejection of claims 1-6 as obvious in view of the disclosure of 5,811,238 issued to Stemmer et al. (hereinafter referred to as the '238 patent) in combination with Berger (Analytical Biochemistry 222: 1-8, 1994). A rejection based on 35 U.S.C. § 103(a) is proper only when the invention as a whole

is shown to be obvious in view of the prior art. Every claim recitation must be considered in determining obviousness over the prior art. In re Boe, 184 U.S.P.Q. 38 (CCPA 1974).

In connection with the rejection under §103, the Examiner indicates that Applicants' remarks describing certain experiments which reveal surprising advantages conferred by separately digesting single stranded DNA were not presented in compliance with the requirements of MPEP 716.01(c). Accordingly, Applicants' hereby submit a declaration under 37 C.F.R. §1.132 setting forth the unexpected and superior results obtained when using separate populations of plus and minus single stranded DNA as the starting material for the generation of polynucleotides encoding polypeptides having improved function in accordance with the methods of the present invention. The contents of Dr. Borrebaeck's declaration are described hereinbelow.

As previously stated in the response to the Official Action of June 29, 2001, the '238 patent to Stemmer discloses several methods of generating variant polynucleotides in vitro, the methods comprising either:

1. fragmenting a mixture of template double-stranded parent polynucleotides, adding to the resultant population of double stranded fragments one or more single or double stranded oligonucleotides having regions of identity to the parent polynucleotide, denaturing the resultant mixture of double stranded fragments and oligonucleotides into single stranded fragments, and annealing the single stranded fragments formed thereby to each other (see column 5, line 51 over to column 6, line 36) or,

2. fragmenting double-stranded parent polynucleotides, denaturing the double-stranded fragments formed thereby, and annealing the resultant single stranded

fragments to single stranded template polynucleotide (see column 6, lines 37-45).

Moreover, the '238 patent provides the following description of the term "template polynucleotide":

"The template polynucleotide often should be double-stranded. A double-stranded nucleic acid molecule is **required** to ensure that regions of the resulting single-stranded nucleic acid fragments are complementary to each other and thus can hybridize to form a double-stranded molecule."

(emphasis added) See column 17, lines 5-10.

Notably, all of the working examples in the '238 patent are directed to the cleavage of double stranded polynucleotides as starting material. In contrast, the methods of the present invention call for the digestion of separate populations of plus and minus single stranded DNA to produce populations comprising either plus or minus single stranded fragments. While the use of single stranded DNA is mentioned in passing at column 6, lines 37-45 of the '238 patent, it is clear from this passage of the description that the single stranded fragments are produced by cleavage of double stranded polynucleotides, the resultant double stranded fragments subsequently being denatured to produce the single-stranded fragments:

"...single-stranded fragments resulting from the cleavage and denaturation of the template polynucleotide..."

In light of the foregoing, Applicants respectfully submit that one of skill in the art would not have been motivated upon reading the '238 patent to utilize separate

populations comprising either plus or minus single stranded DNA as the starting material in methods for generating polynucleotide variants as claimed in the instant application.

As described in the response to the Official Action of June 29, 2001, Berger describes a new class of restriction endonucleases which are referred to as hapaxotermistic (hapaxomer) enzymes. Hapaxomers cleave **double stranded DNA** outside the recognition site or within an interrupted "palindrome" at bases which are not specified, and thus produce fragments with asymmetric, staggered ends (*emphasis added*). The specificity of hexaxomers for double stranded DNA is illustrated in Figure 2 and Table I of the Berger reference. Berger is silent regarding the use of exonucleases in methods to generate polynucleotide variants.

In view of the above, one of ordinary skill in the art would not have been motivated to combine the methods of the '238 patent issued to Stemmer with those of Berger to arrive at the improved method of the present invention. Indeed, if a skilled artisan were to have combined the use of a hexaxomer with the methods of the '238 patent, they would, by definition, have used double stranded DNA as the parental polynucleotide. Applicants, therefore, respectfully submit that the Examiner's position with regard to the obviousness of the present invention in view of the '238 patent and the Berger reference is inappropriate.

In further support of Applicant's position regarding the non-obviousness of the presently claimed methods over the combined disclosures of Stemmer and Berger, the declaration by Dr. Carl Borrebaeck describes the unexpected benefits of using single stranded polynucleotide populations as starting material for DNA shuffling. The advantages of digesting single stranded, as opposed to

double stranded, DNA in shuffling methods are discussed in the declaration and illustrated in Exhibit B attached thereto. The present inventors have made the surprising discovery that the use of single stranded DNA in the methods of the instant application results in increased frequencies of recombination as evidenced by increased numbers of functional, recombined end-products. The frequency of recombined products increases from ~40% to ~60% when single stranded polynucleotide populations, rather than double stranded polynucleotide populations, are used as the source of parent polynucleotide (See Exhibit B). Since improved recombination frequencies are key to the generation of a population of recombined end-products having enhanced diversity from which an optimized end-product may be selected, such an increase provides considerable benefits for experimental productivity. This increase in recombined end-products is not taught or suggested in the '238 patent, alone or in combination with Berger. In view of all of the above, Applicants respectfully submit that the rejection of claims 1-6 as amended under 35 U.S.C. §103 (a) is untenable and should be withdrawn.

The versatility of the presently claimed method is underscored by additional experiments in which different exonucleases at varying concentrations and combinations thereof have been used to generate fragments of a diverse range of sizes for subsequent recombination. The compatibility of the present method with different exonucleases, including: BAL 31, Exo I, T7 gene 6, and Exo V, confers a significant advantage to the method because it enables the generation of populations of polynucleotide fragments of essentially any size range. Accordingly, the size range of a population of single stranded polynucleotide fragments may be tailored to optimize the frequency of functional recombinants generated therefrom.


MISCELLANEOUS

It is respectfully requested that the amendments presented herewith be entered in this application, since the amendments are primarily formal, rather than substantive in nature. This amendment is believed to clearly place the pending claims in condition for allowance. In any event, the claims as presently amended are believed to eliminate certain issues and better define other issues which would be raised on appeal, should an appeal be necessary in this case.

CONCLUSION

The present communication is completely responsive to the issues raised in the Official Action of April 16, 2002. Applicants believe that the claims as they stand are in condition for ready allowance. In the event the Examiner is not persuaded as to the allowability of any claim, and it appears that any outstanding issues may be resolved through a telephone interview, the Examiner is requested to telephone the undersigned attorney at the phone number given below.

Respectfully submitted,
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Enclosures: Marked-up copy of amended claims

Declaration submitted by Dr. Borrebaeck

Exhibits A-B

Marked-up draft of claims

1. A method for generating a polynucleotide sequence or population of sequences from parent single stranded polynucleotide sequences encoding one or more protein motifs, comprising the steps of:

a) providing single stranded polynucleotide sequences [DNA] constituting plus and minus strands of parent polynucleotide sequences;

b) digesting the single stranded [single-stranded] polynucleotide sequences with an exonuclease to generate a first population of single stranded fragments comprising fragments generated from the plus strands and a second population of single stranded fragments comprising fragments generated from the minus strands, said second population being separate from said first population [populations of single stranded fragments];

c) contacting said first population of single stranded fragments [generated from the plus strands] with said second population of single stranded fragments, whereby annealing occurs between said first population of single stranded fragments and said second population of single stranded fragments [generated from the minus strands];

d) amplifying the fragments that anneal to each other to generate at least one polynucleotide sequence encoding one or more protein motifs having altered characteristics as compared to the one or more protein motifs encoded by said parent polynucleotides.

3. A method as claimed in Claim 1 [2] wherein the exonuclease is BAL31.